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Cynthia A. Lemere
Harvard Medical School

Jiwon Oh
Harvard Medical School

Heather A. Stanish
Harvard Medical School

Ying Peng
Harvard Medical School

Imelda Pepivani
Harvard Medical School

See next page for additional authors

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Cerebral Amyloid-Beta Protein Accumulation with Aging in Cotton-Top Tamarins: A Model of Early Alzheimer's Disease?

Cynthia A. Lemere,[1*] Jiwon Oh,[1,2*] Heather A. Stanish,[1,3] Ying Peng,[1] Imelda Pepivani,[1] Anne M. Fagan,[4] Haruyasu Yamaguchi,[5] Susan V. Westmoreland,[6] and Keith G. Mansfield[6]

ABSTRACT

Alzheimer's disease (AD) is the most common progressive form of dementia in the elderly. Two major neuropathological hallmarks of AD include cerebral deposition of amyloid-beta protein (Aβ) into plaques and blood vessels, and the presence of neurofibrillary tangles in brain. In addition, activated microglia and reactive astrocytes are often associated with plaques and tangles. Numerous other proteins are associated with plaques in human AD brain, including Apo E and ubiquitin. The amyloid precursor protein and its shorter fragment, Aβ, are homologous between humans and non-human primates. Cerebral Aβ deposition has been reported previously for rhesus monkeys, vervets, squirrel monkeys, marmosets, lemurs, cynomologous monkeys, chimpanzees, and orangutans. Here we report, for the first time, age-related neuropathological changes in cotton-top tamarins (CTT, *Saguinus oedipus*), an endangered non-human primate native to the rainforests of Colombia and Costa Rica. Typical lifespan is 13–14 years of age in the wild and 15–20+ years in captivity. We performed detailed immunohistochemical analyses of Aβ deposition and associated pathogenesis in archived brain sections from 36 tamarins ranging in age from 6–21 years. Aβ plaque deposition was observed in 16 of the 20 oldest tamarins (>12 years). Plaques contained mainly Aβ42, and in the oldest animals, were associated with reactive astrocytes, activated microglia, Apo E, and ubiquitin-positive dystrophic neurites, similar to human plaques. Vascular Aβ was detected in 14 of the 20 aged tamarins; Aβ42 preceded Aβ40 deposition. Phospho-tau labeled dystrophic neurites and tangles, typically present in human AD, were absent in the tamarins. In conclusion, tamarins may represent a model of early AD pathology.

[1]Center for Neurologic Diseases, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts.
[2]University of Toronto, Toronto, Canada.
[3]Connecticut College, New London, Connecticut.
[4]Washington University School of Medicine, St. Louis, Missouri.
[5]Gunma University Medical School, Gunma, Japan.
[6]New England Regional Primate Center and Harvard Medical School, Boston, Massachusetts.
*These authors contributed equally to this study.

INTRODUCTION

Alzheimer's disease (AD) is the most common form of dementia in the elderly, with prevalence increasing with age. The two major hallmarks of the disease include extracellular amyloid-β (Aβ) deposition into plaques within the limbic and association cortices in brain and the presence of neurofibrillary tangles (NFT) containing hyper-phosphorylated tau and paired helical filaments (PHF).[1] Aβ is formed when the precursor protein (APP) is proteolytically cleaved by β- and γ-secretases generating 40 or 42 amino acid products, known as Aβ40 and Aβ42, respectively.[2] In humans, deposition of Aβ42-immunoreactive (IR) diffuse non-fibrillar plaques precedes deposition of Aβ40 into more compacted plaques while vascular amyloid is more often Aβ40-IR.[3,4] Neuritic plaques contain extracellular Aβ surrounded by dystrophic neurites that are often immunopositive for APP, PHF, phosphorylated tau proteins, and/or ubiquitin. Reactive astrocytes can be found surrounding the perimeter of the amyloid plaque and activated microglial cells are often detected within and surrounding the core.

Although the past several decades of research have dramatically improved our understanding of the pathophysiology of AD, there is still much to be learned about the pathogenesis, risk factors, and pathologic mechanisms underlying this devastating disease. Much of what we know about the disease has been revealed through the pathologic analysis of postmortem human AD brain. In addition, transgenic mouse models overexpressing a human familial AD mutant APP gene and/or presenilin gene (in part responsible for the enzymatic cleavage of the C-terminus of Aβ) have been useful in the understanding of AD pathogenesis and experimental testing of novel therapies.[5] Wild-type mice do not develop cerebral Aβ plaques. In contrast, many non-human primates naturally develop Aβ plaques due to the highly conserved APP sequence between human and non-human primate APP.[6] However, plaque deposition occurs late in non-human primates. Cerebral amyloid-beta deposition has been reported previously for a number of NHP species, including rhesus monkeys, squirrel monkeys, lemurs, marmosets, cynomologous monkeys, chimpanzees, orangutans, and vervets.[6–19] NFTs are absent in most non-human primates; however, plaque-associated degenerating neurites stained by silver or immunoreactive with antibodies raised against APP and phosphorylated neurofilament have been observed in non-human primates.[6–8,11,12,19,20]

The cotton-top tamarin (CTT, *Saguinus oedipus*) is a small (400–500 g) neotropical primate native to Northwestern Columbia that has been used in biomedical research since the early 1970s. Tamarins are arboreal primates that live in extended family units and consume a variety of fruits, insects, and small mammals as a staple of their diet. Widespread habitat destruction and trapping of animals has led to a rapid decline in CTT population numbers and they are listed as a critically endangered species by the Convention on International Trade in Endangered Species (CITES). As with other members of the Callitrichinae, adaptation to the neotropical environment has led to a number of important physiological and disease susceptibility differences from old world primates. Of particular interest is the restricted diversity observed at major histocompatibility class I sites that has been identified in both captive and wild CTT populations.[21,22] In addition, CTT are normally born as dizygotic twins, and anastomosis between placental circulations early in pregnancy leads to stable bone marrow chimerism between twin sets.[23] The roles these factors play in the CTT's unique disease susceptibility pattern is unknown.

In captivity, CTTs routinely live 20 or more years and eventually succumb to a variety of conditions, including diabetes mellitus, carcinoma of the colon, and chronic renal disease. A form of inflammatory bowel disease mimicking ulcerative colitis of man historically has been widespread in tamarin colonies and is believed to be multifactorial in etiology.[24,25] Genetics, dietary factors, environmental stressors, and bacterial pathogens are all believed to play a role in disease phenotype.[26–29] Affected animals develop chronic to intermittent diarrhea, accompanied by weight loss secondary to episodic neutrophilic colitis. This spontaneously occurring condition has been used extensively to investigate novel therapeutic

strategies including the use of humanized monoclonal antibodies directed at key proinflammatory mediators of colonic inflammation such as TNF-α.[30] Repeated episodes of colitis predispose aged animals to the development of colonic adenocarcinoma.[26]

In this study, we report the first detailed immunohistochemical analysis of Aβ deposition, gliosis, neuritic changes, and plaque-associated proteins in the brains of new world cotton-top tamarins ranging in age from 6 to 21 years.

MATERIALS AND METHODS

Primate groups

The autopsied brains of 36 cotton-top tamarins, ranging in age from 6 to 21 years, were examined. The archived samples were provided by the New England Regional Primate Center. Animals were housed in a large breeding colony in accordance with Harvard Medical School's Institutional Animal Care and Use Committee.

Tissue preparation

Blocks of frontal cortex, temporal cortex/hippocampus, and/or occipital cortex from each tamarin were fixed in neutral buffered formalin from 1 to 4 weeks. After fixation, the brain tissues were dehydrated and embedded in paraffin. Sections (10 μ thick) were cut and baked at 60°C for 1 h.

Antibodies and histological stains

All antibodies used for immunohistochemistry are described in Table 1. Each antibody was tested on formalin-fixed, paraffin-embedded human AD brain sections in order to determine optimal staining conditions. A rabbit polyclonal antibody, R1282, that recognizes multiple Aβ forms was used to detect diffuse and compacted plaques and vascular amyloid. Carboxy-terminal specific Aβ42 and Aβ40 mouse monoclonal antibodies, MBC-42 and MBC-40, were used to detect Aβ ending at residues 42 and 40, respectively. Anti-glial fibrillary acid protein (GFAP) was used to detect reactive astrocytes while anti-Iba-1 was used to stain activated microglia. An anti-APP monoclonal antibody, 8E5, that detects APP residues 444–592 was used to detect APP fragments in dystrophic neurites within neuritic plaques. Anti-Apo-E was used to detect apolipoprotein E in amyloid plaques. Anti-ubiquitin was used to detect dystrophic neurites in plaques while an anti-phospho-tau monoclonal antibody, AT8, was used to detect NFTs and neuritic dystrophy. Routine thioflavin S staining was performed to detect fibrillar amyloid.

Table 1. Antibodies Used for Immunohistochemistry

Antibody	*Target*	*Species*	*Dilution*	*Pretreatment*	*Source*
R1282	Aβ (general)	rabbit anti-human	1:1000	formic acid	D. Selkoe (Boston, MA)
MBC-40	Aβ-40	mouse anti-human	1:1000	formic acid	H. Yamaguchi (Gunma, Japan)
MBC-42	Aβ-42	mouse anti-human	1:1000	formic acid	H. Yamaguchi
GFAP	Reactive Astrocytes	rabbit anti-human	1:1000	none	DAKO (Carpenteria, CA)
Iba-1	Activated Microglia	rabbit anti-human	1:200	microwave	WAKO (Richmond, VA)
Apo-E	Apo-E	goat anti-human	1:1000	formic acid, microwave	Chemicon (Temecula, CA)
AT8	NFTs, dystrophic neurites	mouse anti-human	1:25	microwave	Innogenetics (Belgium)
Ubiquitin	NFTs, dystrophic neurites	rabbit anti-human	1:5000	none	East Acres Biologicals (Southbridge, MA)
22C11	APP	mouse anti-human	1:1000	microwave	Chemicon (Temecula, CA)

Immunohistochemistry

Sections were deparaffinized in Histoclear (National Diagnostics, Atlanta, GA) and rehydrated in a graded series of ethanols. Incubating the sections in 0.3% hydrogen peroxide in methanol for 5 min at room temperature quenched endogenous peroxidase activity. After washing the sections in water for 5 min, appropriate pretreatments for each primary antibody were applied, as described in Table 1. Microwave pretreatment entailed heating sections in the microwave at high power in citrate buffer (Biogenex, San Ramone, CA) until the buffer came to a boil, at which point the heat level was reduced in order to provide cyclic boiling for an additional 6 min. The sections were cooled to room temperature and washed in several changes of water. Formic acid pretreatment consisted of applying 88% formic acid to the sections for 15 min, followed by two 5 min washes in water. Following pretreatments, all sections were blocked for 20 min in 10% goat serum (GS), 10% horse serum (HS), or 5% Carnation dried non-fat milk in TBS-Tween (10 mM Tris [pH 8], 0.15 M NaCl, 0.05% Tween-20). Sections were incubated with primary antibodies overnight at 4°C. The horseradish peroxidase (HRP) avidin-biotin complex system (rabbit, mouse, or goat Elite ABC kits; Vector Laboratories, Burlingame, CA) and diaminobenzidine (DAB, Sigma Immunochemicals, St. Louis, MO) were used to visualize bound antibodies. In order to reduce run-to-run variability, sections from all tamarins were stained with a given antibody simultaneously. Sections were then counterstained with hematoxylin, dehydrated, cleared in Histoclear (National Diagnostics), and cover slipped with Permount (Fisher Scientific, Pittsburgh, PA). As a negative control, primary antibody was omitted from a single section during immunostaining with each antibody, consistently resulting in a lack of immunoreactivity.

Quantification of serum Aβ by ELISA

Frozen aliquots of serum were obtained for 27 of the 36 tamarins. Serum levels of Aβ1-40 and Aβ1-42 were quantified by sandwich ELISA at Washington University School of

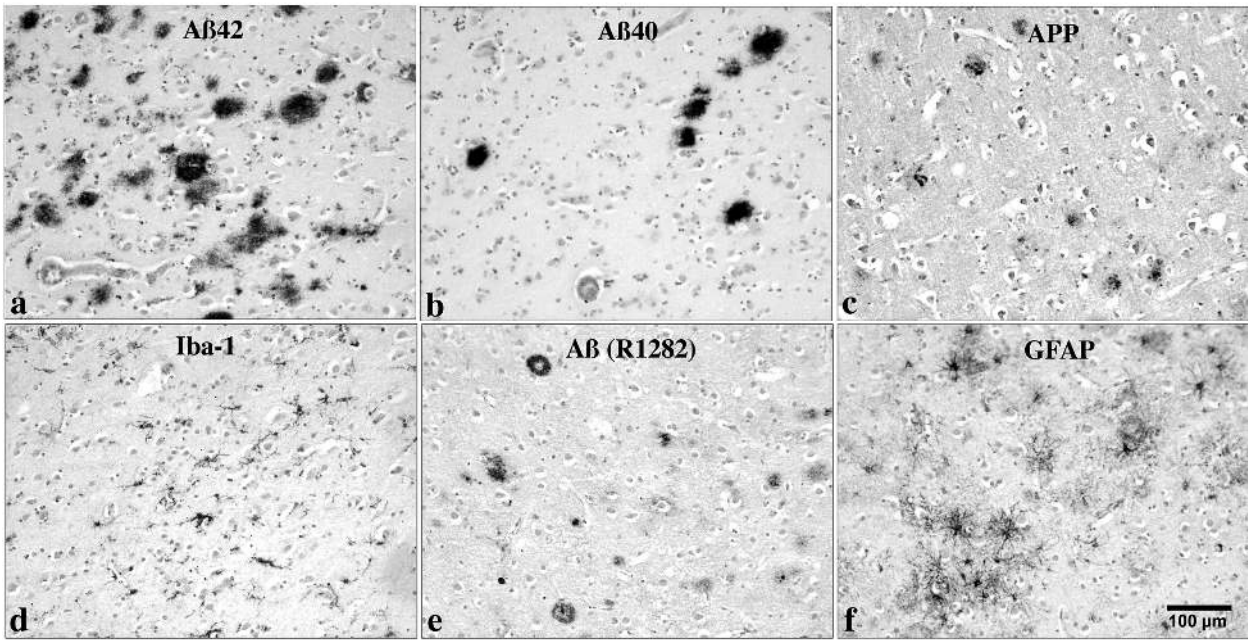

FIG. 1. Human AD neuropathology. Immunohistochemistry was used to detect Aβ deposition and accompanying neuropathological changes in formal-fixed paraffin sections from the frontal cortex of an 80-year-old female AD patient. Abundant diffuse and compacted plaques were detected with anti-Aβ42 (**a**), while only a subset of plaques, mostly compacted, was labeled with anti-Aβ40 (**b**). A general Aβ antibody, R1282, labeled a subset of Aβ42-immunoreactive plaques (**e**). Neuritic plaques were identified by labeling of dystrophic neurites with anti-APP 8E5 (**c**). Activated microglia (**c**) and reactive astrocytes (**f**) were increased in areas containing compacted plaques. Scale, 100 μm.

Medicine using C-terminal specific antibodies 2G3 and 21F12, respectively, to capture and a biotinylated N-terminal specific antibody, 3D6, to detect, as described.[31] All samples were run in triplicate and compared with two serum samples from human controls.

RESULTS

Human AD pathology

As shown in Figure 1, human AD is characterized by the presence of extracellular Aβ plaques (Fig. 1a, b, e) that often contain APP-positive dystrophic neurites (Fig. 1c) and are surrounded by reactive astrocytes (Fig. 1f) and activated microglia (Fig. 1d). Aβ42 deposition is found in both diffuse, non-fibrillar plaques and compacted, fibrillar plaques and is more abundant than Aβ40, found primarily in a subset of compacted plaques and vascular deposits. In addition, NFTs are present and immunoreactive with antibodies against certain phosphorylated forms of tau and neurofilament proteins and ubiquitin (data not shown). Thioflavin S labels fibrillar amyloid in compacted plaques, meningeal and parenchymal blood vessels, and NFTs (data not shown).

Cerebral Aβ deposition in aged tamarins

Cortical brain tissues from 36 tamarins (ages 6–21 years; 21 females, 15 males) were examined by immunohistochemistry for AD pathology, as illustrated in Table 2. While both frontal

Table 2. Immunoreactivity in Tamarin Cortex

Tamarin ID	*Age (yr)*	*Gender*	*Aβ: R1282*	*Aβ42*	*Aβ40*	*GFAP*	*Iba-1*	*Apo E*	*Ubiquitin*
161-96	6.3	F	–	–	–	–	–	–	–
29-96	6.53	F	–	–	–	–	–	–	–
288-93	6.6	M	–	–	–	–	–	–	–
83-96	6.7	M	–	–	–	–	–	–	–
25-92	6.9	M	–	–	–	–	–	–	–
301-94	7.7	F	–	–	–	–	–	–	–
307-91	7.9	F	–	–	–	–	–	–	–
462-92	8.1	F	–	–	–	–	–	–	–
59-93	8.4	M	–	–	–	–	–	–	–
328-90	8.7	F	–	–	–	–	–	–	–
350-92	8.8	M	–	–	–	–	–	–	–
136-92	9.4	M	–	–	–	–	–	–	–
234-90	9.8	M	–	–	–	–	–	–	–
*12-90	10.5	M	–	–	–	–	–	–	–
106-87	11.2	M	–	–	–	–	–	–	–
15-88	11.3	M	–	–	–	–	–	–	–
499-91	12.4	F	+bv	+bv	–	–	–	+bv	–
496-92	13.7	F	–	+pl	–	–	–	–	–
202-89	14.5	F	+bv	+bv	–	–	–	+bv	–
295-92	14.5	M	+pl	+pl	–	–	–	–	–
297-84	15.6	F	+pl; +bv	+pl; +bv	–	–	–	+pl; +bv	–
102-83	15.7	F	–	+pl	–	–	–	–	–
382-82	16.2	M	+pl	+pl	–	–	+	–	–
402-87	16.2	F	+bv	+bv	–	–	–	–	–
60-88	16.6	F	+pl; +bv	+pl; +bv	+pl	+	+	–	–
151-88	16.9	F	+pl	+pl	–	–	–	–	–
245-86	16.9	F	+pl; +bv	+pl; +bv	–	–	–	+bv	–
383-85	16.9	F	+bv	+bv	–	–	+	–	–
271-88	17.3	F	+pl	+pl	–	+	+	–	–
234-84	17.4	F	+pl; +bv	+pl; +bv	–	–	+	–	–
62-85	17.4	F	+pl	+pl; +bv	–	+	+	+bv	–
29-81	19.5	M	+pl; +bv	+pl; +bv	–	+	+	–	+
160-80	19.6	M	+pl; +bv	+pl; +bv	+bv	+	+	+pl	–
159-80	20.2	F	+pl; +bv	+pl; +bv	+bv	+	+	+pl; +bv	+
203-84	20.8	M	+pl; +bv	+pl; +bv	+pl; +bv	+	+	+pl; +bv	+
87-79	20.9	F	+pl; +bv	+pl; +bv	+bv	+	+	+pl; +bv	+

F, female; M, male; bv, blood vessels; pl, plaques.

and temporal cortical samples were available for most of the tamarins, occipital samples were obtained from a subset of tamarins. Cerebral Aβ deposition was observed first in blood vessels starting at 12 years of age (Fig. 2a) and then in plaques beginning at 13 years of age. Gender did not influence the age of onset of Aβ deposition (Table 2). While some diffuse, granular Aβ deposits were observed (Fig. 2b), many plaques were rounded and appeared compacted (Fig. 2c-f). The number of plaques was much greater in the oldest animals ($\geq$19 yrs). In general, Aβ plaque and vascular deposits occurred first in frontal and temporal cortices; however, vascular Aβ deposition was also present and much more abundant in occipital cortex.

Plaques were detected in hippocampus in only the oldest animals and in very low numbers. Therefore, most of the data presented here pertains to cortical regions of tamarin brain. Thioflavin S labeled fibrillar Aβ in a subset of blood vessels and compacted plaques (data not shown).

Aβ42 deposition precedes Aβ40 deposition in plaques and vascular amyloid in aged tamarins

Sensitive C-terminal-specific antibodies were used to detect Aβ ending at residues 42 (Aβ42) and 40 (Aβ40) in tamarin brain. Similar to human brain, Aβ42-positive plaques were observed earlier than Aβ40-positive plaques in tamarin brains (Table 2 and Fig. 3). Aβ42-positive plaques were observed in 16 of 20 tamarins over 12 years of age. Both diffuse (Fig. 3b) and compacted plaques (Fig. 3a and c) were immunoreactive with the Aβ42 monoclonal antibody. In contrast, Aβ40-positive plaques were observed only in two tamarins (ages 16.6 and 20.8 years), and were few in number and found only in compacted plaques (data not shown).

As illustrated in Table 2 and Figure 4, vascular Aβ deposition was comprised mainly of Aβ42, with Aβ40-immunoreactive blood vessels occurring only in the four oldest animals (ages 19.6–20.9 years) and predominantly in occipital cortex. Strong Aβ42-positive vascular

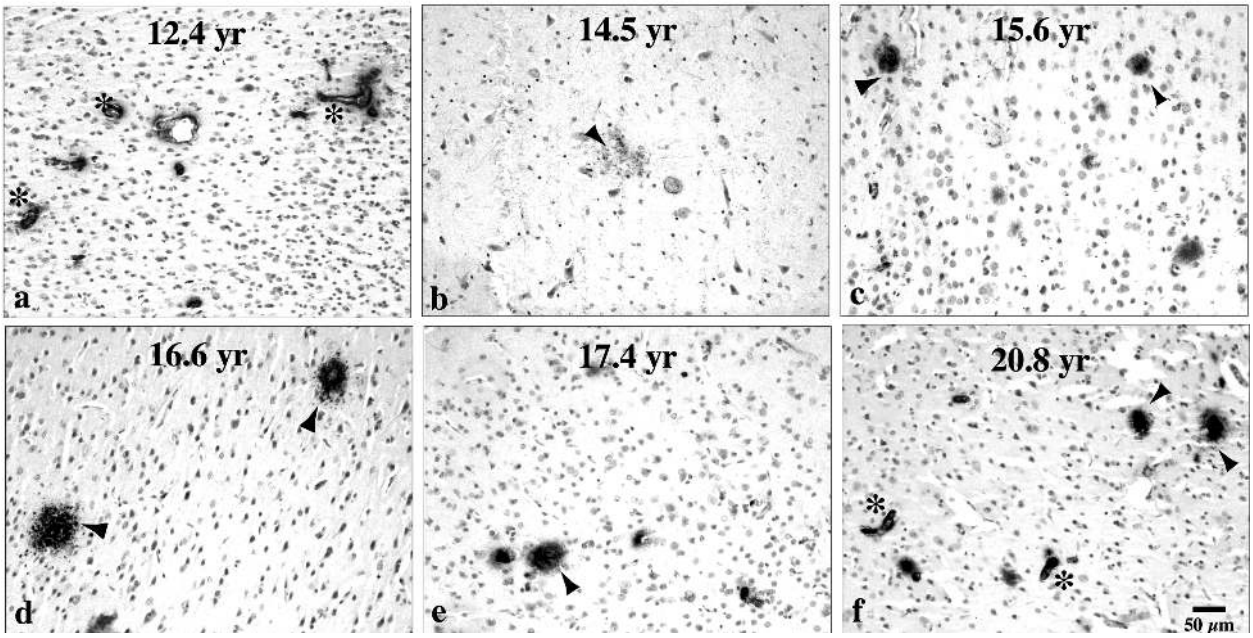

FIG. 2. Aβ Immunoreactivity in frontal cortex of tamarin. A general Aβ antibody, R1282, was used to immunostain tamarin frontal cortex sections. Vascular Aβ deposits (*) were observed as early as 12 years of age (**a**) and increased in abundance with aging (**f**). Diffuse granular plaques (**b**) are seen in the younger of the aged animals, whereas more rounded, compacted plaques as well as diffuse plaques were observed in the older animals (**c–f**, arrows). Scale, 50 μm.

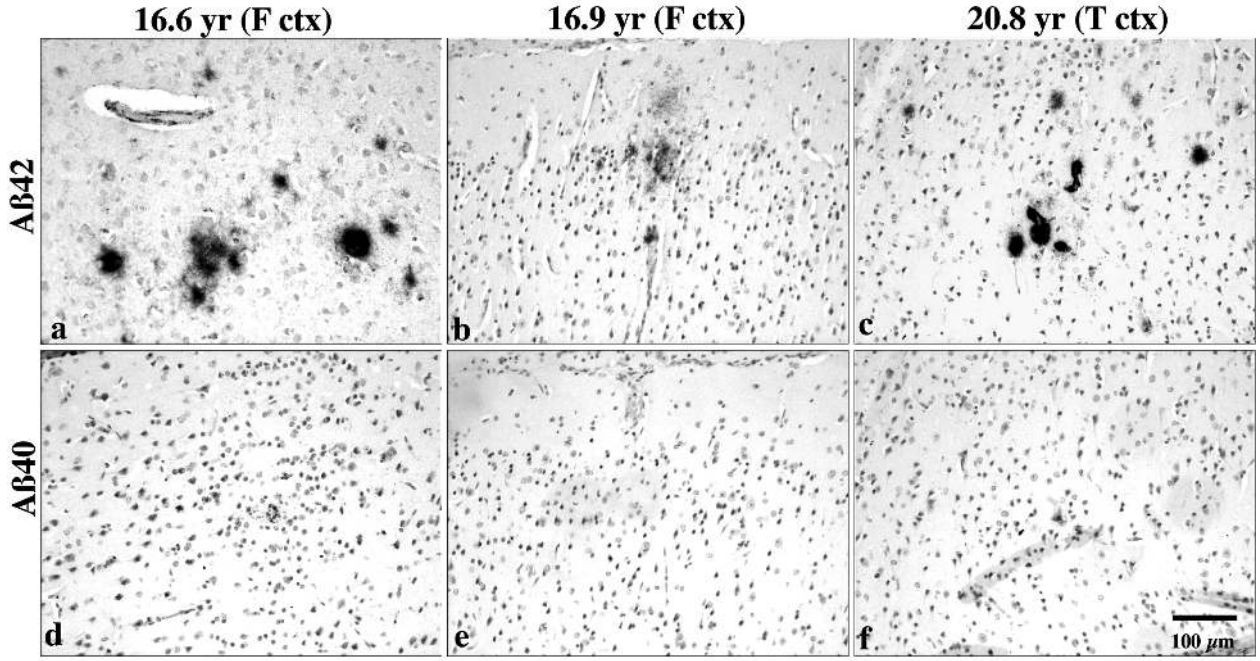

FIG. 3. Aβ42 precedes Aβ40 in cerebral plaques in tamarins. Monoclonal antibodies recognizing the free-carboxyl terminus of Aβ ending at residue 42 or 40 were used to immunostain formalin-fixed, paraffin brain sections of tamarin. Aβ42 immunoreactivity (**a–c**) was observed earlier and in much greater quantity than Aβ40 immunoreactivity (**d–f**) in plaques in adjacent serial sections. Aβ42 labeling was found in diffuse, granular deposits (**b**) as well as in compacted plaques (**a, c**). Aβ40 immunoreactivity was mostly absent from the adjacent sections, except for two small dots in the center (**f**). F ctx, frontal cortex; T ctx, temporal cortex. Scale bar, 100 μm.

amyloid was observed at 12.4 years of age in frontal and occipital cortices in the absence of any Aβ40 immunoreactivity (Fig. 4a and d). Aβ42 deposition was detected in leptomeningeal (Fig. 4a and b) as well as parenchymal blood vessels in aged tamarins (Fig. 4c). Thioflavin S labeled most of the vascular amyloid (data not shown), indicating the presence of Aβ fibrils.

Plaque-associated pathology in tamarin brain

Gliosis, Apo E, and ubiquitin were examined by immunohistochemistry in tamarin brain sections. Anti-GFAP immunolabeled astrocytes in all tamarin brain sections; however, plaque-associated reactive astrocytes were detected in cortex in eight aged tamarins (16.6–20.9 years; Table 2) (data not shown in image). Plaque-associated activated microglia were detected in cortex by Iba-1 immunolabeling in 11 aged tamarin brains (16.2–20.9 years; Table 2 and Fig. 5a and b). Gliosis was also prominent around blood vessels containing amyloid (data not shown). Apo E, a plaque-associated protein found in human AD brain, was detected in amyloid-laden blood vessels as early as 12.4 years of age and in subset of cerebral plaques beginning at 15.6 years of age in tamarin cortex (Table 2 and Fig. 5b and c). However, many Aβ plaques did not have any Apo E immunoreactivity. Lastly, plaque-associated dystrophic neurites were detected in cortex using an anti-ubiquitin antibody in four of five of the oldest animals (Table 2 and Fig. 5d and f) although no neuritic plaques were observed using anti-APP (8E5) and anti-phospho-tau (AT8) antibodies (data not shown).

Serum Aβ levels in tamarins

Stored frozen serum samples were obtained for 27 (ages 6.3–20.8 years) of the 36 tamarins examined neuropathologically in this study. All samples were subjected to Aβ1–42 and Aβ1–40 ELISAs. In general, serum Aβ40 and Aβ42 levels were markedly lower in all 27 tamarins compared to two control human serum samples. Aβ40 levels averaged 17.2 pg/mL (± 29.8 SD) for tamarins and 391.5

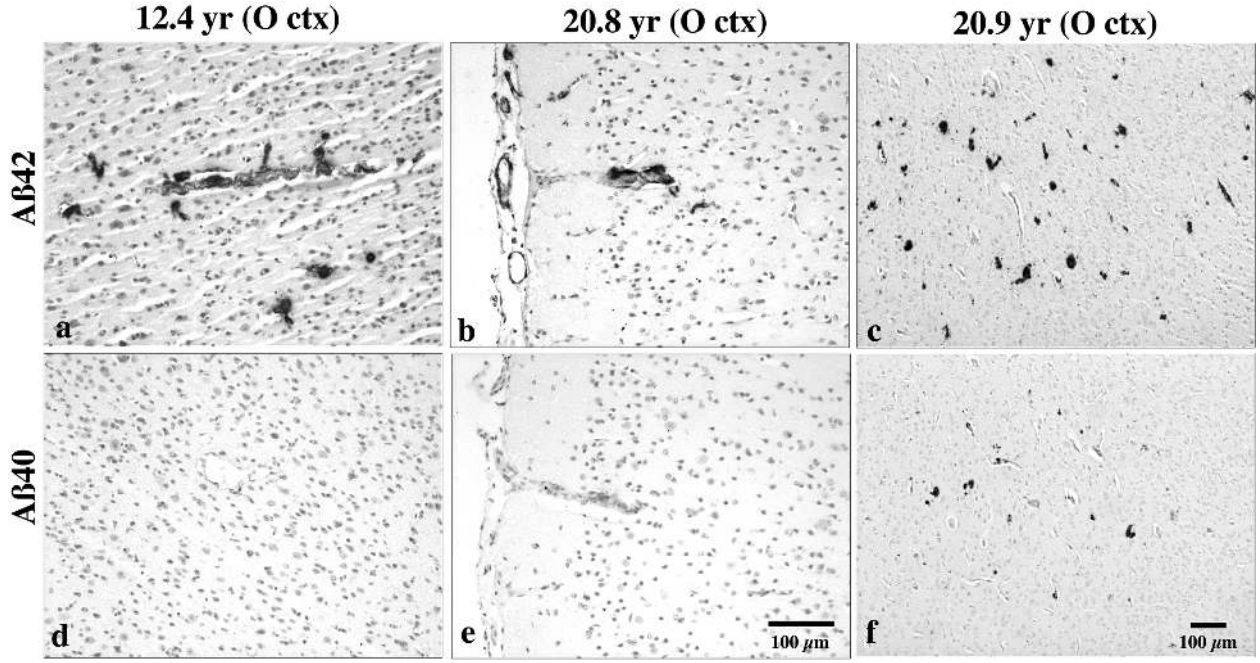

FIG. 4. Aβ42 precedes Aβ40 in cerebral blood vessels in tamarins. Monoclonal antibodies recognizing the free-carboxyl terminus of Aβ ending at residue 42 or 40 were used to immunostain formalin-fixed, paraffin brain sections of tamarin. Aβ42-immunoreactive blood vessels were observed earlier (**a**) and in much greater quantity (**a–c**) than Aβ40-immunoreactive blood vessels (**d–f**) in adjacent serial sections. Aβ deposition occurred in both leptomeningeal (**b**) and parenchymal blood vessels, although in both types of vessels, Aβ42 was the dominant species at all ages. O ctx, occipital cortex. Scale bar (**e**), 100 μm; scale bar (**f**) for **c and f** only, 100 μm.

pg/mL (± 31.6 SD) for humans. Aβ42 levels averaged 15.0 pg/mL (± 11.8 SD) for tamarins and 43.7 pg/mL (± 39.2 SD) for humans. Interestingly, the levels of Aβ40 and Aβ42 were roughly equal in tamarin serum while Aβ40 was approximately 9-fold higher than Aβ42 in human serum. Aβ levels in serum did not correlate with Aβ deposition in plaques or blood vessels in tamarin brain.

Lack of correlation between Aβ deposition and colitis in tamarins

As mentioned earlier, cotton-top tamarins frequently develop ulcerative colitis. Because colitis is an inflammatory-based illness, we asked whether animals with colitis were more likely to develop Aβ deposition. Eleven of the 16 tamarins under 12 years of age were reported to have colitis at the time of death; cerebral Aβ deposition was absent in these animals. Ten of 20 tamarins 12 years of age or older had colitis but all 20 of these animals displayed some cerebral Aβ immunoreactivy in plaques, blood vessels, or both. Thus, there was no correlation between colitis and the amount of Aβ deposition in animals 12 years of age or older.

DISCUSSION

Animal models, such as cotton-top tamarins, that naturally deposit Aβ into plaques and blood vessels in brain provide a useful tool for understanding the pathogenesis of AD, and may help to identify novel biomarkers for early diagnosis. In addition, these models represent valuable resources for preclinical testing of therapeutic strategies for AD, although such testing would preclude terminal endpoints in tamarins as they are an endangered species. Here, we show that with aging (beginning around 12 years of age), tamarins develop both vascular amyloid and cortical Aβ plaques, both of which contain predominantly Aβ42 protein. Diffuse and compacted plaques were observed in frontal, temporal, and occipital cortices; however only the more compacted plaques were associated with gliosis, Apo E, and ubiq-

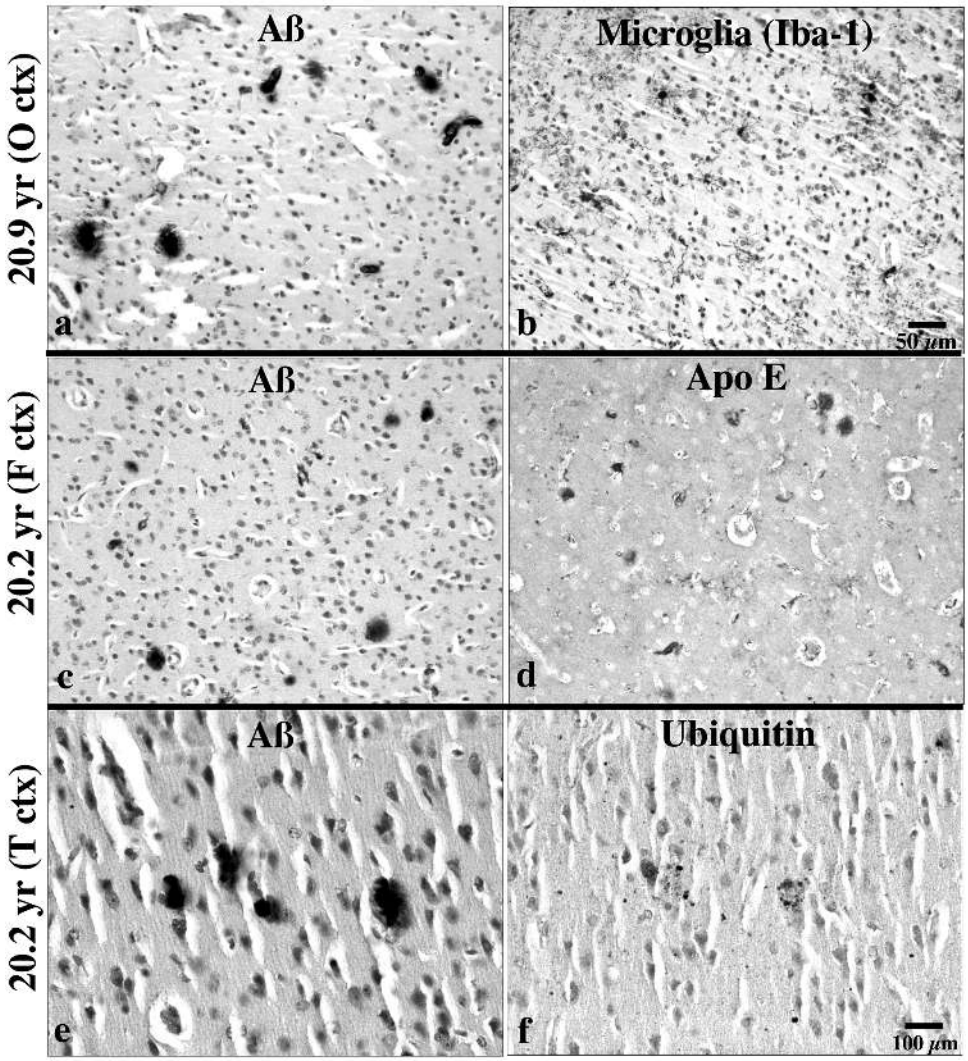

FIG. 5. Plaque-associated pathology in tamarin brain. Cortical sections were immunostained using antibodies to microglia (Iba-1), apolipoprotein E (Apo E, a cholesterol transport protein thought to play a role in Aβ deposition), and ubiquitin. Plaque-associated activated microglia were occasionally observed (**a and b**) in 11 of the 20 oldest animals and increased with age. Apo E staining co-localized with Aβ in a subset of cortical plaques (**c and d**) and/or blood vessels in 9 of the 20 oldest tamarins. Ubiquitin-positive dystrophic neurites were observed infrequently in Aβ plaques (e and f), and only in four of the five oldest animals (>19 years). Scale bar (**a–d**), 50 μm; scale bar (**e and f**), 100 μm.

uitin-positive dystrophic neurites. Phospho-tau-positive and APP-positive neuritic plaques and NFTs were not observed in any of the 36 tamarin brains examined in this study. Aβ was detectable in low levels in serum but did not correlate with Aβ deposition in brain. Colitis, a common inflammatory affliction in tamarins, did not appear to accelerate or increase Aβ pathology in tamarin brain.

Although cerebral Aβ deposition was noted in a few canine species as early as 1956,[32] it was not until the 1970s that the observation of cerebral Aβ deposition came to include various non-human primate species.[33] Although canine species were found to have Aβ plaques in brain parenchyma, few of the plaques were found to progress to characteristic full-blown AD pathology as that seen in humans.[34] More recently, the development of AD-like transgenic mouse models has allowed for many advances in the understanding of AD pathophysiology and has concurrently provided a convenient model upon which to test therapies.[5] However, mice do not naturally develop Aβ pathology, possibly due to a three amino acid difference in the first 15 residues of human versus murine

Aβ. Because of substantial genetic, biochemical, and physiological differences between rodents and humans, it is not surprising that the use of murine models for the experimental testing of novel therapies has its limitations, as was exemplified when an AD vaccine that was clearly efficacious in clearing cerebral Aβ deposits in mouse models caused serious complications (aseptic meningoencephalitis) in a Phase II clinical trial in humans.[35] Hence, it is apparent that these two animal models are useful but each has its own limitations.

Non-human primates provide a more natural model of AD-like pathology, as they develop Aβ plaques and cerebrovascular amyloid pathology with aging, and have a highly conserved APP sequence compared to humans.[6] A body of accumulated research indicates that the neuropathological consequences of aging in non-human primates is almost indistinguishable from what occurs in humans.[36] Furthermore, due to their vast repertoire of behavioral habits, non-human primates provide a useful model to document and compare the behavioral effects of various therapies. Amyloid deposition in both cerebral parenchyma and vasculature have previously been observed in various primate species, including squirrel monkeys, marmosets, lemurs, rhesus monkeys, vervets, cynomolgus monkeys, chimpanzees, and orangutans.[6–20] Of these, the rhesus monkey and squirrel monkey have been among the most extensively studied thus far. C-terminal specific antibodies have been used in many of these species in order to elucidate the type and distribution of Aβ deposition. Our data confirm the prevalence of Aβ42 in plaques and blood vessels in tamarins, similar to humans and some published data in non-human primates,[13] with the exception that in some non-human primate species, plaque and vascular amyloid consist mainly of Aβ40.[15] In part, this may be due to the age of the animals investigated (Aβ42 is deposited earlier than Aβ40 in tamarins) and the antibodies and pretreatments used in the different studies. The presence of neuritic dystrophy, reactive astrocytosis, activated microglia, as well as various plaque-associated proteins such as α 1-ACT, Apo-E, and heparin sulfate proteoglycan have also been observed in various primate species.

Serum Aβ levels were much lower in tamarins than in humans. It is possible that the Aβ antibodies used in the ELISA are less efficient at detecting tamarin Aβ than human Aβ but this seems unlikely as similar Aβ antibodies were able to detect extracellular Aβ in tamarin brain tissue. It is also possible that Aβ is bound to another protein or is in a particular conformation in tamarin serum, making it less accessible to the Aβ antibodies. Further studies are underway to address these possibilities. In addition, it is unclear why Aβ40 and Aβ42 levels are similar in tamarins while Aβ40 is much higher than Aβ42 in humans. It is possible that this finding may be relevant to the greater abundance of Aβ42 in vascular Aβ in tamarins compared to humans.

In summary, we have described naturally occurring Aβ pathology in the brains of aged cotton-top tamarins. While Aβ deposition, particularly Aβ42, was present in plaques and blood vessels in the cortex of numerous tamarins after 12 years of age, more advanced pathological changes, such as the presence of Aβ40 immunoreactivity, gliosis, Apo E deposition, and neuritic dystrophy, was evident only in the oldest animals. Neurofibrillary tangles were not observed using one phospho-tau antibody, AT8. Our observations indicate that cotton-top tamarins develop early AD-like pathology similar to that seen in humans, and would thus be a useful model of early AD pathology and possibly biomarkers for early diagnosis.

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Address reprint requests to:
Cynthia A. Lemere, Ph.D.
NRB636F
77 Avenue Louis Pasteur
Boston, MA 02115

E-mail: clemere@rics.bwh.harvard.edu